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Pentachlorophenol Toxicity to Amphipods and Fathead Minnows at Different Test pH Values

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16. ABSTRACT

Juvenile amphipods (Gammarus pseudolimnaeus and Crangonyx pseudogracilis) and fathead minnows (Pimephales promelas) were exposed to pentachlorophenol (PCP) at pH values of 6.5, 7.5, 8.0 and 8.5 for 96 h, and early life stages of fathead minnows were exposed for 32 d at the same pH values to determine the relationship between test pH and the acute and chronic toxicity of PCP. Residue analyses were performed on fathead minnows after 32 d to determine the relationship between test pH and PCP bioaccumulation. Technical-grade PCP (88% active ingredient; Dowicide EC7) was used as the toxicant source.

Acute exposures with all three species showed that PCP toxicity was decreased with increased test pH. The slopes of this relationship were similar for the amphipods, but were different from that observed for fathead minnows. Early life stage exposures of fathead minnows showed that chronic PCP toxicity and bioaccumulation were similarly decreased when pH values were increased. The decrease in chronic PCP toxicity appeared to be due to the reduction in PCP accumulation as a direct result of the increased ionization of PCP at higher pH values, indicating that the ionized form was less toxic per unit concentration than was the un-ionized form. However, these studies suggest that chronic as well as acute toxicity may be caused by both forms of PCP when significant concentrations are present in solution.

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PENTACHLOROPHENOL TOXICITY TO AMPHIPODS AND FATHEAD MINNOWS AT DIFFERENT TEST pH VALUES

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Abstract—Juvenile amphipods (*Gammarus pseudolimnaeus* and *Crangonyx pseudogracilis*) and fathead minnows (*Pimephales promelas*) were exposed to pentachlorophenol (PCP) at pH values of 6.5, 7.5, 8.0 and 8.5 for 96 h, and early life stages of fathead minnows were exposed for 32 d at the same pH values to determine the relationship between test pH and the acute and chronic toxicity of PCP. Residue analyses were performed on fathead minnows after 32 d to determine the relationship between test pH and PCP bioaccumulation. Technical-grade PCP (88% active ingredient; Dowicide EC7) was used as the toxicant source.

Acute exposures with all three species showed that PCP toxicity was decreased with increased test pH. The slopes of this relationship were similar for the amphipods, but were different from that observed for fathead minnows. Early life stage exposures of fathead minnows showed that chronic PCP toxicity and bioaccumulation were similarly decreased when pH values were increased. The decrease in chronic PCP toxicity appeared to be due to the reduction in PCP accumulation as a direct result of the increased ionization of PCP at higher pH values, indicating that the ionized form was less toxic per unit concentration than was the un-ionized form. However, these studies suggest that chronic as well as acute toxicity may be caused by both forms of PCP when significant concentrations are present in solution.

Keywords—Pentachlorophenol Toxicity Bioconcentration pH Invertebrates Fish

INTRODUCTION

Pentachlorophenol (PCP) and its sodium salt (NaPCP) have been used extensively in agriculture and industry because of their versatility. Collectively they are the second most used pesticide in the United States [1], and because of their high solubility in solvents and water have been used in a wide variety of industrial processes. The most common use of PCP in the United States and Canada is for wood preservation [1,2].

As a result of its wide application, PCP has been detected in human and other terrestrial animal tissues [3], and in aquatic systems and organisms [4]. Numerous studies have indicated that PCP is toxic to aquatic plants and animals [5–10]. Early studies by Crandall and Goodnight [11] and more recent work by Kobayashi and Kishino [12], Kishino and Kobayashi [13] and Saarikoski and Viluksela [14] have also indicated that physical variables, most notably pH, can influence the toxicity of PCP in water. Results of these studies showed that acute PCP toxicity and bioaccumulation were increased as the pH of the water was decreased. The importance of this relationship is apparent as the acidification of aquatic systems continues due to industrial pollution, mainly from acid mine drainage and, in the longer term, from acid precipitation. The effect of acidification on aquatic life and on the toxicity of some chemicals has been illustrated [15–19]. No studies, however, were found in the literature on the relationship between test pH and the chronic toxicity and accumulation of PCP in aquatic animals.

The purpose of this study was to compare the relationship between toxicity and test pH for both invertebrates and fish. Acute toxicity tests were conducted with amphipods (*Gammarus pseudolimnaeus* and *Crangonyx pseudogracilis*) and fathead minnows (*Pimephales promelas*), and chronic toxicity and bioaccumulation were estimated using 32-d early life stage tests for fathead minnows. The

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application of 32-d early life stage toxicity tests for fish in predicting chronic toxicity has been established [20].

MATERIALS AND METHODS

Water characteristics

Lake Superior water was filtered through sand and heated to 22 ± 2°C for acute tests and 25 ± 2°C for 32-d early life stage tests with fathead minnows. Chemical characteristics of the dilution water for tests conducted at four pH values were determined according to methods described by the American Public Health Association [21]. The ranges for water hardness, alkalinity and acidity were 42 to 47, 10 to 52 and 0 to 9 mg/L (as $CaCO_3$) for all tests, respectively. The range for free CO_2 was 0.32 to 6.32 mg/L. Alkalinity, acidity and CO_2 concentrations varied with the pH of the test water. Dissolved oxygen concentrations were >60% saturation in all tests, and the range was 6.6 to 8.7 mg/L. The pH of the test water was monitored continuously with a Corning Model 12 pH meter and a recorder. Mean pH values and SD calculated from individual measurements recorded weekly from 32-d tests with fathead minnows are included in Table 1. Mean pH values for acute tests with amphipods and fathead minnows were similar to those of the 32-d tests. Mean pH was determined by converting pH values to their corresponding hydrogen ion concentration, averaging these values and then reconverting the averages to pH units.

Exposure system

Tests were conducted with continuous-flow minidiluter exposure systems [22] that delivered five PCP concentrations and a control to four replicate chambers per treatment. Duplicate exposure chambers per treatment were used in the acute toxicity tests due to the smaller number of animals available. Glass test chambers measured 7 cm wide × 19 cm long × 9 cm high, with a water depth of 4.5 cm. The flow rate to each chamber was 15 ± 1 ml/min. Sylvania Cool White fluorescent bulbs provided a light intensity of 100 to 300 lux at the water surface during a 16-h photoperiod.

Toxicant solution

Technical-grade PCP (88% active ingredient; Dowicide EC7) was supplied by Dow Chemical (Midland, Michigan) and used as the toxicant source. Superstocks were made by dissolving PCP with NaOH in distilled water. Diluter stocks were also prepared with distilled water in 19-liter glass bottles and were delivered to the diluter systems via fluid-metering pumps to produce the desired test concentrations. The desired pH was obtained and maintained by pumping dilute solutions of either HNO_3 or NaOH into aerated dilution water located in head tanks throughout the tests.

Chemical procedures

Water samples for PCP analyses were collected twice weekly from each treatment, acidified with 0.10 ml H_2SO_4, and (1:5, v/v) and extracted with 50 ml hexane. Sample size varied according to expected concentrations. The samples were mixed for ~1 h to ensure complete extraction and 1.0-ml samples were derivatized with 0.10 ml diazomethane. The chemical analyses were performed with a Hewlett-Packard 5730A gas chromatograph equipped with a Model 3552A data system and a ^{63}Ni electron capture detector. The column was packed with 100/120 mesh Supelcoport coated with 1.5% SP2250/1.95% SP2401. The carrier gas was argon containing 5% methane. Analyses were run at a column temperature of 165°C. The limit of detection for this procedure was ~0.30 μg/L.

Table 1. Acute 96-h LC50 values (μg/L) of PCP for juvenile amphipods and fathead minnows at four pH values[a]

Species	pH			
	6.5 ± 0.3	7.5 ± 0.1	8.0 ± 0.2	8.5 ± 0.2
Amphipod (*Gammarus pseudolimnaeus*)	92 (65–132)	121 (77–192)	484 (359–652)	790 (585–1,067)
Amphipod (*Crangonyx pseudogracilis*)	139 (98–197)	465 (323–668)	929 (723–1,194)	1,344 (692–2,608)
Fathead minnow (*Pimephales promelas*)	95 (77–116)	218 (169–280)	261 (197–346)	378 (307–464)

[a]95% confidence intervals in parentheses.

The mean percentage recovery and SD for 10 spiked samples were 98 ± 6.5, 99 ± 4.1, 101 ± 2.4 and 100 ± 2.8 for tests conducted at pH 6.5, 7.5, 8.0 and 8.5, respectively. Measured water concentrations for tests conducted at various pH are included in Table 2.

Biological procedures

Testing began in March and ended in November 1982. Both 96-h acute and 32-d early life stage tests conducted at pH 6.5, 7.5, and 8.0, 8.5 were run simultaneously for each species. Procedures for conducting acute tests generally followed those described by the American Society for Testing and Materials [23]. Flow-through acute toxicity tests were begun by distributing 10 (~30-d old) juvenile fathead minnows or 10 (0.73–17 mg) amphipods to each treatment. Acute LC50 values were determined from tests lasting 96 h. Control survival was 90% or greater in all tests.

Early life stage tests with fathead minnows were begun by randomly distributing 25 <24-h-old embryos from our culture unit into 120-ml glass jars with 40-mesh Nytex screen bottoms. The jars were oscillated in the exposure chambers with a 2 rpm motor to determine hatchability of the embryos. After 2 d of incubation, the number of developed embryos was randomly thinned to 15 in each replicate chamber (60 embryos per treatment) to eliminate embryo mortality due to fungus. Subsequently, these embryos were incubated until they hatched (~4–5 d). After all embryos were hatched,

Table 2. Survival and growth of fathead minnows exposed to various concentrations of PCP for 32 d at pH values of 6.5, 7.5, 8.0 and 8.5

Measured water concentration (µg/L)	Embryo hatchability[a] (%)	Normal larvae at hatch (%)[a]	Survival[a] (%)	Mean weight[b] (mg)
pH = 6.5				
<0.3[c] (control)	100 ± 0.0	100 ± 0.0	91 ± 6.7	81 ± 20(54)
3.1 ± 0.3[d]	98 ± 3.5	98 ± 3.5	96 ± 4.0	74 ± 20(58)
5.1 ± 0.4	100 ± 0.0	100 ± 0.0	91 ± 10.1	80 ± 20(43)
8.9 ± 0.9	100 ± 0.0	100 ± 0.0	95 ± 6.7	74 ± 20(58)
16.5 ± 1.8	100 ± 0.0	100 ± 0.0	95 ± 6.7	74 ± 17(56)
34.6 ± 2.4	100 ± 0.0	0[e]	23 ± 8.8[e]	88 ± 37(14)
pH = 7.5				
<0.3[c] (control)	100 ± 0.0	95 ± 6.7	90 ± 4.0	98 ± 28(41)
5.6 ± 0.6[d]	100 ± 0.0	95 ± 6.7	91 ± 10.1	103 ± 28(39)
12.7 ± 1.4	100 ± 0.0	93 ± 9.4	88 ± 11.5	102 ± 24(52)
27.6 ± 4.2	100 ± 0.0	100 ± 0.0	98 ± 3.5	90 ± 26(59)
58.2 ± 7.6	100 ± 0.0	88 ± 6.3	84 ± 6.1	81 ± 30(50)[e]
124.0 ± 6.4	82 ± 6.7[e]	0[e]	0[e]	—
pH = 8.0				
<0.3[c] (control)	100 ± 0.0	97 ± 4.0	88 ± 10.0	100 ± 24(53)
12.7 ± 2.6[d]	98 ± 3.5	97 ± 7.0	88 ± 10.0	108 ± 24(53)
32.0 ± 4.3	100 ± 0.0	95 ± 6.7	91 ± 3.6	109 ± 32(54)
75.0 ± 5.8	95 ± 6.3	93 ± 5.7	92 ± 10.1	90 ± 26(54)[e]
161.0 ± 6.1	95 ± 3.5	0[e]	29 ± 19.3[e]	88 ± 41(18)[e]
327.0 ± 13.4	54 ± 22.3[e]	0[e]	0[e]	—
pH = 8.5				
<0.3[c] (control)	100 ± 0.0	97 ± 4.0	93 ± 5.1	108 ± 31(56)
29.3 ± 2.3[d]	100 ± 0.0	100 ± 0.0	93 ± 5.7	104 ± 35(55)
63.7 ± 4.4	98 ± 3.5	97 ± 4.0	92 ± 10.1	107 ± 28(52)
125.0 ± 6.1	100 ± 0.0	100 ± 0.0	75 ± 22.8[e]	104 ± 30(44)
237.0 ± 16.0	100 ± 0.0	0[e]	75 ± 14.7[e]	88 ± 41(45)[e]
451.0 ± 33.8	82 ± 16.7[e]	0[e]	13 ± 0.0[e]	7 ± 0.0(1)[e]

[a]Mean of 15 organisms per replicate.
[b]Number of fish weighed in parentheses.
[c]Detection limit of analyses.
[d]Mean ± SD of 8 to 10 samples.
[e]Values significantly less than controls ($p = 0.05$).

the larvae were transferred to the exposure chambers by gently inverting the egg cups in the dilution water until all larvae were released. All animals were fed ad libitum amounts of brine shrimp nauplii three times per day, except on weekends when they were fed once daily. After a total of 32 d of exposure, the juvenile fish were killed in ice water, blotted dry and each fish was weighed to the nearest milligram.

Residue analysis

Fathead minnows that survived the 32-d tests were analyzed on a whole body basis (wet weight) for PCP. Fish from each replicate chamber were composited and analyzed as one sample. The fish were placed in 125-ml glass-stoppered bottles containing 50 ml of concentrated H_2SO_4 and were digested in an oven at 90°C for approximately 2 h. After cooling and the addition of 50 ml hexane, the samples were extracted, derivatized and analyzed in the same manner as the water samples. The limit of detection for this procedure was <0.1 μg/g. The mean percentage recovery and SD for five spiked samples were 96 ± 3.7.

Bioconcentration factors (BCFs) were calculated by dividing the concentration of PCP in whole fish tissue by the corresponding measured water concentrations at no effect levels.

Statistical analyses

Ninety-six-hour LC50 values were determined with a computerized, modified trimmed Spearman-Karber method described by Hamilton et al. [24]. Daily mortality data of replicate exposure tanks were combined before LC50 values were calculated.

For early life stage tests with fathead minnows, survival and embryo hatchability data were transformed to arc sin % [25] for variance stabilization. Individual weights from fish in replicate chambers were pooled (after no significant differences were found between replicates) before the data were subjected to a one-way analysis of variance ($p = 0.05$) and Dunnett's one-sided comparison of treatment means to control means ($p = 0.05$) [26].

Linear regression analysis (Minitab) [27] was used to describe the relationship between PCP toxicity and accumulation versus pH. Analysis of covariance [28] was used to determine slope comparability.

RESULTS AND DISCUSSION

The acute (96-h) LC50 values for amphipods and fathead minnows exposed to PCP at four pH values are presented in Table 1. Comparison of the values for amphipods indicate that *G. pseudolimnaeus* was approximately two times more sensitive than *C. pseudogracilis* at corresponding pH values. Fathead minnow sensitivity was similar to that of amphipods at pH 6.5 and 7.5, but was appreciably greater at the higher pH values. In other studies, Kaila and Saarikoski [29] and Hedtke and Arthur [30] found that fish are more acutely sensitive to PCP than are several species of invertebrates, including amphipods.

The relationship between the log LC50 values of PCP and pH for amphipods and fathead minnows is illustrated in Figure 1. The direct correlation between these values and pH indicate that acute PCP toxicity was reduced as the pH was increased. Good correlations were observed for the regression lines, especially for *C. pseudogracilis* and fathead minnows ($p < 0.05$). A slightly lower correlation for *G. pseudolimnaeus* ($r^2 = 0.85$, $p < 0.10$) was attributed to the lower than expected value (higher toxicity) at pH 7.5. The relationship between pH and the toxicity of PCP to these species agrees with earlier work on fathead minnows [11] and other fish species [12,14], as well as for invertebrates [29] exposed to this chemical at various pH values. Studies by Kobayashi and Kishino [12] and Saarikoski and Viluksela [14] indicated, however, that this relationship may not be linear at extreme pH where more abrupt effects on toxicity were observed. Analysis of covariance of the regression lines in Figure 1 indicates that the slopes for the relationship with amphipods were not significantly different from each other, but that a significant difference was noted when the slopes were compared with that for fathead minnows. A similar relationship between test pH and PCP toxicity to amphipods was expected because these animals are taxonomically similar and the mode of toxic action of PCP is probably the same in each. The difference in this relationship for fathead minnows, however, suggested that the toxicity of PCP as a function of test pH may affect various animal groups differently. Furthermore, the greater slopes for amphipods would indicate that acute toxicity as related to test pH may be greater in invertebrates than in fish. This hypothesis is supported by comparisons of results between other invertebrates and fish exposed to phenols [31].

Survival and growth of fathead minnows exposed to different concentrations of PCP for 32 d and to four pH values are shown in Table 2. Embryo hatchability was consistently the least

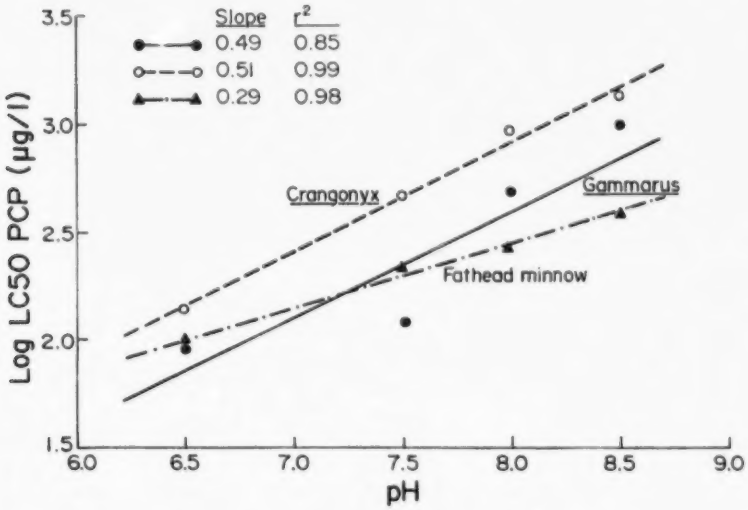

Fig. 1. Relationship between log 96-h LC50 values of PCP and pH for acute tests with amphipods and fathead minnows.

sensitive measure of effect in all tests. Although fish hatched in all concentrations, morphological and behavioral abnormalities such as scoliosis, pericardial edema and convulsive swimming patterns were observed in larvae exposed to the highest concentrations. Survival of the larvae was the most sensitive measure of PCP effect at pH 6.5 and 8.5, whereas growth, as measured by wet weight, was the most sensitive parameter measured in tests at pH 7.5 and 8.0. Chronic values defined as the geometric mean of the upper chronic limit (the lowest test concentration that caused significant decreases from the control) and the lower chronic limit (the highest test concentration that did not cause significant decreases from the control) based on the above results were 24, 40, 49 and 89 μg/L at the respective increasing pH values. The chronic values of 40 and 49 μg/L for pH 7.5 and 8.0 compare closely to a value of 57 μg/L calculated from the chronic limits (45.9–73 μg/L) for fathead minnows exposed to PCP at pH 7.2 to 7.9 [10].

The relationship of the log chronic values to pH and to the log of the distribution ratio ([PCP$^-$]/[PCP]) of PCP in water is illustrated in Figure 2. Chronic values increased as both the pH and the distribution ratio increased. Correlations for both relationships were $r^2 = 0.95$ ($p < 0.05$). It can be shown that the log of the ratio of ionized to un-ionized PCP concentration is related to the pH of the solution and the pK_a of PCP by

$$\log \frac{[PCP^-]}{[PCP]} = pH - pK_a \qquad (1)$$

The pK_a for PCP is 4.74 [32]. Because pK_a in Eqn. 1 is a constant, the relationship between the log of the chronic values and the log distribution ratio is the same as that between log chronic value and pH, indicating that the chronic toxicity in terms of total PCP decreased as the concentration of ionized PCP and pH increased. This can also be seen if the acute 96-h values (Fig. 1) are plotted against this ratio. Because the pH values in our studies are above the pK_a value for PCP, the ionic form of PCP represents >98% of the total PCP present in each of the pH tests (from Eqn. 1). Thus, the change in the ratio of the ionized to the un-ionized form of PCP with pH appears to be due primarily to changes in the proportion of the un-ionized form. The increase in total PCP required to obtain chronic values at higher pH values (Fig. 2) apparently results, then, from the need to replace un-ionized PCP with the ionized form as the pH is increased. This was similarly concluded by Kishino and Kobayashi [13] for acute tests with goldfish exposed to PCP at various pH values. These results suggest that the ionized form

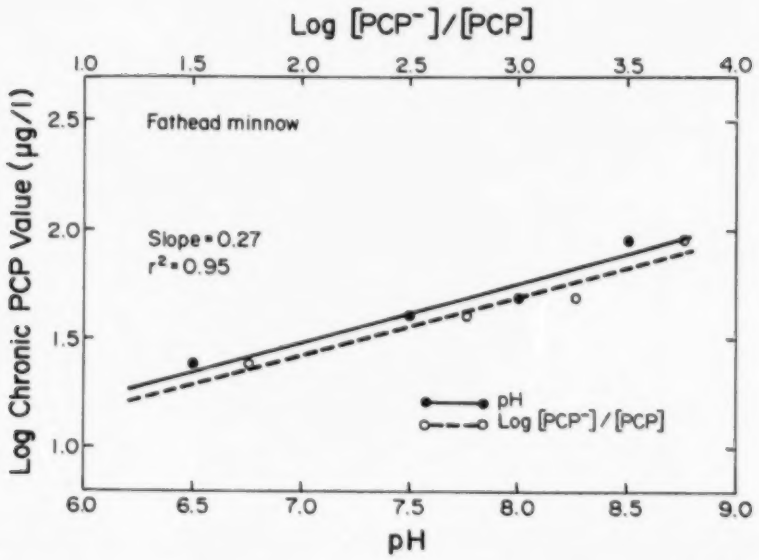

Fig. 2. Relationship between log chronic PCP values and pH or log [PCP⁻]/[PCP] for fathead minnows exposed for 32 d.

of PCP is probably much less toxic per unit concentration than the un-ionized form on a chronic basis as well as in acute tests. Analysis of covariance of our regression lines for the acute and chronic values versus the distribution ratio demonstrated that the slopes (0.29 and 0.27, respectively) were not significantly different from each other, indicating that the acute and chronic toxicities of PCP were similarly affected by changes in the test pH. However, additional long-term tests with other species are needed to describe this relationship before the effect of pH on chronic PCP toxicity can be predicted on the basis of acute tests.

The accumulation of PCP in fathead minnows after 32 d of exposure at four pH values is presented in Table 3. Concentrations of PCP in fish whole body increased proportionally with an increase in exposure at both no effect and effect concentrations. BCFs for these data were 1,066, 434, 426 and 281 for the respective increasing pH values. The relationship between BCFs and pH is illustrated in Figure 3. A correlation of $r^2 = 0.94$ ($p < 0.05$) shows that BCFs decreased with a rise in the pH of the test water. This result agrees with that observed for goldfish acutely exposed at various pH values [12]. Because the un-ionized form of chlorophenols can penetrate through membranes faster than the ionized form [31,33], the decrease in accumulation of PCP in fathead minnows at higher pH appears to be due to the change in chemical form of PCP from the un-ionized to the ionized form (Fig. 2), as was proposed previ-

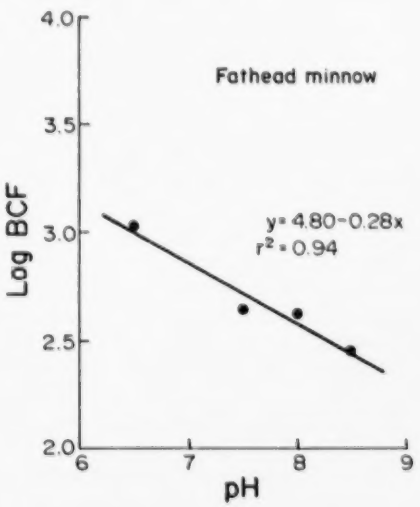

Fig. 3. Relationship between log bioconcentration factor (BCF) and pH for fathead minnows exposed to PCP for 32 d.

Table 3. Accumulation of PCP in whole-body fathead minnows after 32 d of exposure at four pH values

pH = 6.5		pH = 7.5		pH = 8.0		pH = 8.5	
Water	Tissue	Water	Tissue	Water	Tissue	Water	Tissue
<0.3[a]	<0.1[a]	<0.3	<0.1	<0.3	<0.1	<0.3	<0.1
3.1 ± 0.3[b]	2.8 ± 0.3	5.6 ± 0.6	2.5 ± 0.1	12.7 ± 2.6	5.8 ± 0.1	29.3 ± 2.3	8.3 ± 0.8
5.1 ± 0.4	5.0 ± 0.5	12.7 ± 1.4	5.2 ± 0.4	32.0 ± 4.3	12.6 ± 1.1	63.7 ± 4.4	17.8 ± 1.2
8.9 ± 0.9	9.6 ± 0.2	27.6 ± 4.2	12.3 ± 0.5	75.0 ± 5.8	22.1 ± 3.2	125.0 ± 6.1	35.1 ± 4.6
16.5 ± 1.8	21.5 ± 5.0	58.2 ± 7.6	25.1 ± 1.7	161.0 ± 6.1	43.8 ± 8.9	237.0 ± 16.0	69.0 ± 6.4
34.6 ± 2.4	45.9 ± 12.7	124.0 ± 6.4	—[c]	327.0 ± 13.4	—	451.0 ± 33.8	—

Measured exposure concentrations are expressed in μg/L and tissue concentrations in μg/g (wet weight).
[a]Control at detection limit of analysis.
[b]Mean and SD of 8 to 10 samples.
[c]No residues were obtained due to high mortality.

ously by Kobayashi and Kishino [12]. Studies by Kishino and Kobayashi [13] relating the absorption mechanisms of PCP in goldfish to its distribution between solvents and water showed that there is a pH-dependent, linear relationship between BCF and the distribution ratio of PCP in an alcohol/water system. These results demonstrate that the transfer of PCP from the water to the fish is caused mainly by passive diffusion of the un-ionized form, probably through the gill membrane. These findings are consistent with the relationship between the log *n*-octanol/water partition coefficient (log *P*), which measures relative lipophilicity and BCF [34] and between log *P* and pH [35]. Toxicity and the mechanism of uptake and elimination of chlorophenols in fish and their relationship to log *P*, pK_a and pH has been discussed by Könemann and Musch [33] and Saarikoski and Viluksela [31].

Because both the toxicity and accumulation of PCP decreased with increased pH in these studies, the reduction in both acute and chronic toxicity in fish at higher values of pH appears to result from the fish not accumulating PCP to concentrations that cause adverse effects as readily as at low pH. Concentrations of PCP in dead fathead minnows averaged approximately 75 μg/g, as compared with the highest residue concentration of 35 μg/g in fish not adversely affected by PCP after 32 d of exposure. In comparison, goldfish began to die when PCP concentrations in the body reached a similar value of 92 μg/g body weight, an effect independent of the variation in the pH of the test water [12].

Although un-ionized PCP is more readily accumulated than the ionized form, ionized PCP can accumulate slightly by hydrogen-bonding to constituents that make up biological membranes [13]. Thus, the toxic action of PCP appears to be caused by the ionized as well as the un-ionized form [14,33]. This can be seen in part by the relationship between chronic toxicity, expressed in terms of un-ionized PCP, and pH (Fig. 4). The concentration of un-ionized PCP can be obtained from the anti-log of Eqn. 1 as

$$\frac{[PCP^-]}{[PCP]} = 10^{pH - pK_a} \quad (2)$$

and the mass balance of PCP in water given by

$$\text{total}[PCP) = [PCP) + [PCP^-] \quad (3)$$

Rearrangement and substitution of Eqns. 2 and 3 gives

$$[PCP] = \frac{\text{total } [PCP]}{(1 + 10^{pH - pK_a})} \quad (4)$$

A correlation of $r^2 = 0.99$ ($p < 0.05$) shows that the log of chronic values in terms of un-ionized PCP decreased as the pH of the water increased. Because the concentration of ionized PCP increases with increasing pH (Fig. 2), the decrease in values (Fig. 4) suggests that the chronic toxicity of un-ionized PCP increases per unit concentration with the rise in pH, and/or that the ionized form is toxic to some degree. Saarikoski and Viluksela [14] discussed the fact that although both forms of phenol contribute to toxicity, the toxicity of the ionized form decreases with rising pH, indicating that changes in toxicity are caused partially by changes in the degree of ionization and partially by changes in the absorption rate of the ionized form.

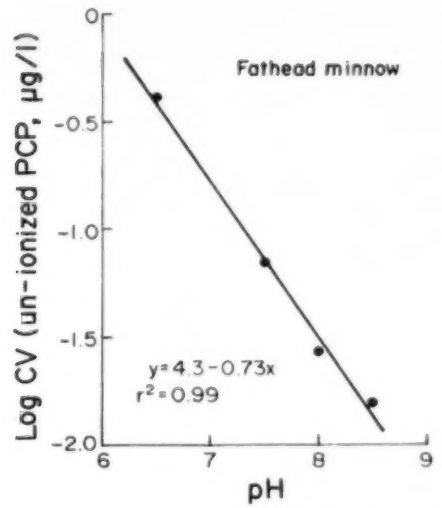

Fig. 4. Relationship between log chronic values (CV) expressed in terms of un-ionized PCP and pH for fathead minnows exposed for 32 d.

The latter agrees with the present results and suggests that ionized PCP is contributing significantly to the overall toxicity of PCP at high pH values where the ionic concentration is much greater than the un-ionized concentration.

CONCLUSIONS

The present study has shown that an increase in the pH of the test water decreased the acute toxicity of PCP to both amphipods and fathead minnows. The slopes of this relationship were similar for the two species of amphipods, but the response observed for fathead minnows appeared less dependent upon pH than that for either species of amphipods.

Comparison of the test results on fathead minnows indicated that pH affected chronic PCP toxicity in a similar manner as it did acute toxicity. The decrease in the chronic toxicity of this chemical to fathead minnows with the rise in pH appears to be due to the reduction in PCP accumulation as a direct result of the increased ionization of PCP in water, indicating that the ionized form is less toxic per unit concentration than is the un-ionized form. This was also postulated in earlier acute toxicity studies with fish. It appears, however, that ionized PCP is contributing significantly to the overall chronic toxicity of PCP when it is present in large concentrations at higher levels of pH.

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